INCURSION OF THE DT104 MULTIRESISTANCE LOCUS INTO *SALMONELLA CHOLERAESUIS*

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Abstract The increasing prevalence of multi-drug resistance in pathogenic bacteria is a significant problem for food safety. *Salmonella enterica* serovar Typhimurium DT104, which is a global health concern and infects a broad range of mammalian hosts, has been shown to carry a chromosomal integron (SGI-1) which encodes multiple antibiotic resistance: ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline (ACSSuT). The portion of this integron encoding antibiotic resistance is 13 kb and the remaining 30 kb is of unknown function but may contribute to hypervirulence in DT104. The SGI-1 antibiotic resistance gene cluster has subsequently been identified in other serovars suggesting lateral transfer of this element. Host-adapted *Salmonella* have been assessed for the SGI-1 integron. Isolates of *S. choleraesuis,* the swine-adapted serovar, with the ACSSuT phenotype have been recovered from pooled clinical isolates. Full sequencing of the SGI-1 is underway. Characterization of this integron provides insight into antibiotic resistance, virulence, and SGI-1 transfer between pathogens.

Introduction Multidrug resistant (MDR) *Salmonella* are a major concern in veterinary medicine, food safety, and agricultural practices. *Salmonella enterica* serovar Typhimurium DT104, which is a global health concern and infects a broad range of mammalian hosts, has been shown to carry a chromosomal integron (SGI-1) which encodes multiple antibiotic resistance: ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline (ACSSuT). This 43 kb integron is composed of a 13 kb region encoding antibiotic resistance adjacent to 30 kb of unknown function. This larger region contains 15 unknown ORFs as well as genes related to mating pair formation and DNA transfer (Boyd *et al.,* 2001) thus suggesting this is mobile element. In addition, genes in SGI-1 may influence the hypervirulence phenotype observed in DT104 as demonstrated by the correlation between poor clinical outcomes and the presence of SGI-1 (Evans and Davies, 1996; Rasmussen *et al*., 2005). Our laboratory has recently identified one of the 15 unknown ORFs, hereby designated SGI-1 virulence ORF (SvO), as an activator of hypervirulence for DT104 exposed to protozoa.

SGI-1 was originally described in DT104 in 1999 (Briggs and Fratamico, 1999). The SGI-1 antibiotic resistance gene cluster has since been identified in *S. enterica* serovars Agona (Boyd *et al.,* 2001; Giraud *et al*., 2000), Paratyphi B (Meunier *et al.,* 2002), Albany (Doublet e*t al.,* 2003), Infantis (Carlson *et al.,* 1999), Meleagridis (Ebner *et al*., 2004), and Newport (Doublet *et al*., 2004), suggesting lateral transfer of this element. In addition, the entire 43 kb integron was recently conjugally transferred *in vitro* between *S. enterica* and an *Escherichia coli* recipient (Doublet *et al.,* 2005). Given this evidence for horizontal and vertical transfer of SGI-1, its occurrence in additional *Salmonella* serovars is likely and is of great relevance for therapeutic applications and food safety.

While SGI-1 has been found in the aforementioned serovars with broad host ranges, this gene cluster has not been observed in host-adapted serotypes such as *Salmonella enterica* serotype Choleraesuis. Herein, we assessed *S. choleraesuis* for the presence of SGI-1. Isolates of *S. choleraesuis* with the ACSSuT phenotype have been recovered from pooled clinical isolates and DNA sequencing of SGI-1 is underway. Characterization of this integron is essential for assessing implications for antibiotic resistance, virulence, and transmission between pathogens.

Materials and Methods Over 300 *Salmonella choleraesuis* clinical and non-clinical isolates were obtained from the National Veterinary Service Laboratories (Ames, IA). Isolates were pooled and grown in Lennox L broth (Difco) with ampicillin (32 µg/ml, Sigma) and florfenicol (15 µg/ml, Schlering-Plough). The presence of SGI-1 was confirmed using PCR with previously described primers (Carlson *et al*., 1999).

Sequencing of SGI-1 was accomplished using Elongase PCR (Invitrogen) to amplify 6-8 kb regions followed by TOPO pCR-XL cloning (Invitrogen). Primers were designed based on the SGI-1 sequence of *S.* Typhimurium DT104. PCR conditions were according to manufacturer's instructions, using the 68° combined annealing and extension. PCR products were visualized using crystal violet and purified by gel excision per manufacturer's instructions. Colonies were screened for

inserts using Eco RI digests and PCR with the M13 primers. Plasmid DNA was isolated using the Plasmid Maxi Kit (Qiagen) and sequenced at the Iowa State University DNA Facility. Sequences checked using BLAST and aligned using BioEdit.

Results ACSSuT *Salmonella choleraesuis* were identified from pooled clinical isolates. Sequencing is currently underway to evaluate identity of SGI-1 in *S. choleraesuis* with other SGI-1 sequences. Comparisons between SGI-1 from DT104 and SGI-1 from *S. choleraesuis* will focus on the antibiotic resistance genes and SvO. Additionally, the SGI-1 insertion site will be identified for the *S. choleraesuis* genome.

Discussion Our results have further extended the distribution of SGI-1 and known MDR *Salmonella*. SGI-1 had not been previously known for any host-adapted *Salmonella*. This finding highlights the transmissibility of this integron and evokes questions regarding the phenotype of those strains containing SGI-1.

Future *in vivo* studies focused on evaluating hypervirulence in these strains will provide insight into the functions of the unknown ORFs. Our recent studies indicate that SvO is activated when DT104 is engulfed and then escapes from rumen protozoa (Rasmussen *et al.*, 2005). While swine are rarely exposed to rumen protozoa, they are exposed to amoeba and other free-living protozoa that could serve as activators of SvO for *S. choleraesuis*. Therefore, future studies will also examine *in vivo* virulence of amoeba-exposed *S. choleraesuis* that have SGI-1. The ubiquity of SvO is another line of research that can provide the basis for understanding the sporadic and anecdotal reports of *Salmonella* hypervirulence.

Conclusions MDR in *Salmonella* is relevant to food safety, veterinary medicine, and agriculture. Evaluating the genetic basis for these phenotypes serves to inform the transmissibility of MDR as well as accompanying virulence features. Increasing sequence data on elements such as SGI-1 provides insight into the evolution of these genetic elements and a basis for predicting epidemiology.

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